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| In re Patent Application of: | ) | |
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| Bengt Krister OLSON | ) | Group Art Unit: To Be Assigned |
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| Serial No.: 09/853,635 | ) | Examiner: To Be Assigned |
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| Filed: May 14, 2001 | ) | |

For: COMBINED MARINE AND PLANT EXTRACT COMPOSITIONS

**SUBMISSION OF CERTIFIED COPY OF PRIORITY DOCUMENT**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicant respectfully submits the certified copy of Denmark Patent Application No. DK PA 2000 00782, filed May 12, 2000, in connection with the above-identified patent application. Applicant claimed foreign priority benefits under 35 U.S.C. § 119 from the Denmark Application in the above-identified Application filed in the U.S. Patent and Trademark Office on May 14, 2001.

Respectfully submitted,

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# Kongeriget Danmark

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This is to certify the correctness of the following information:

The attached photocopy is a true copy of the following document:

- The specification and claims as filed with the application on the filing date indicated above.

Patent- og Varemærkestyrelsen
Kongeriget Danmark

**Patent- og**
**Varemærkestyrelsen**
Erhvervsministeriet

Taastrup 25 May 2001

Karin Schlichting
Head Clerk

# COMPOSITIONS FOR ORAL USE

## FIELD OF THE INVENTION

The present invention concerns compositions for oral use, said compositions containing cartilage or extracts thereof as well as hydrophilic and lipophilic antioxidants.

## BACKGROUND OF THE INVENTION

Free radicals are formed in the body, e.g. in the skin, as a result of UV radiation, pollution, alcohol, etc. Excess of free radicals can cause severe damage to tissue structure, including skin structure, and thus signs of ageing begin to appear.

There has therefore been made use of antioxidants, both hydrophilic and lipophilic in combination, in reducing the oxidative stress caused by free radicals in i.a. the skin [Maffei Facino *et al.* ("Free Radical Scavenging and Anti-enzyme Activities of Procyanidines from Vitis vinifera" Arzneim.-Forsch./Drug Res., **44**(1), Nr. 5 (1994), pp 592-601)].

It has also been established that the administration of protein complexes containing mucopolysaccharides derived from marine cartilage sources have the ability to improve the texture of the dermis of the skin by making it more dense and firm [Kieffer ME, Efsen J., *J. Eur. Acad. Dermatol. Venereol.*, 1998 Sep; 11(2):129-136].

## SUMMARY OF THE INVENTION

In a first aspect, the invention therefore concerns a composition for oral use, said composition comprising

i) cartilage or an extract thereof,

ii) a hydrophilic antioxidant, and

iii) a lipophilic antioxidant exhibiting an antioxidant activity $CI_{50}$ of at least $1.1 \times 10^{-7}$ for scavenging R'/ROO' radicals in lipid peroxidation of an unsaturated phospholipid in an aqueous medium.

## DETAILED DESCRIPTION OF THE INVENTION

In the context of the present invention, the term "hydrophilic" as applied to the hydrophilic antioxidant generally means that the antioxidant is sufficiently soluble in, and hence able to function in, an aqueous medium in the body. In the present context, an antioxidant is considered to be hydrophilic if it has a solubility in water of above 0.05 g per 100 g of water. A hydrophilic antioxidant preferably has a solubility in water of above 0.5 g, more preferably above 1 g, in particular above 5 g, more particularly above 10 g, most particularly above 25 g, especially above 50 g, such as above 100 g per 100 g of water.

Similarly, the term "lipophilic" as applied to the lipophilic antioxidant generally means that it is sufficiently soluble in, and hence able to function in, a lipid medium in the body. This also means that it has a very low solubility in water. In the present context, an antioxidant is considered to be lipophilic if it has a solubility in water of below 0.05 g per 100 g of water. A lipophilic antioxidant preferably has a solubility in water of below 0.005 g, in particular below 0.0005 g per 100 g of water.

As it will appear from the above, the antioxidant properties of the lipophilic antioxidant is expressed as its ability of scavenging R˙/ROO˙ radicals in lipid peroxidation of an unsaturated phospholipid in an aqueous medium. The analysis method in question is described by Maffei Facino *et al.* indicated above ("Free Radical Scavenging and Anti-enzyme Activities of Procyanidines from Vitis vinifera" Arzneim.-Forsch./Drug Res., **44**(1), Nr. 5 (1994), pp 592-601) which is hereby incorporated by reference.

In a preferred embodiment, the antioxidant activity $CI_{50}$ of the lipophilic antioxidant is at least $1.2 \times 10^{-7}$ for scavenging R˙/ROO˙ radicals in lipid peroxidation of an unsaturated phospholipid in an aqueous medium, preferably at least $1.3 \times 10^{-7}$, more preferably at least $1.4 \times 10^{-7}$, particularly at least $1.5 \times 10^{-7}$, more particularly at least $1.6 \times 10^{-7}$, especially at least $1.7 \times 10^{-7}$, such as at least $1.8 \times 10^{-7}$.

In a particularly preferred embodiment, the lipophilic antioxidant exhibiting an antioxidant activity $CI_{50}$ of at least $1.1 \times 10^{-7}$ for scavenging R˙/ROO˙ radicals in lipid peroxidation of an unsaturated phospholipid in an aqueous medium is the carotenoid compound lycopene (also termed ψ,ψ-carotene). Lycopene may typically be obtained by extraction from cer-

tain fresh fruits such as tomatoes, water melon, red grapefruit or guava fruit in a manner known *per se*, or it may be prepared synthetically in a known manner.

The lycopene may optionally be present in combination with another lipophilic antioxidant. The optional further lipophilic antioxidant may be selected from the group consisting of carotenoids, procarotenoids, tocopherols and phytosterols and mixtures thereof. Preferred examples of such optional further lipophilic antioxidants are vitamin E, α-carotene, β-carotene, ubidecarenone, ubiquinol, cryptoxanthine, zeaxanthine and leutine.

The cartilage or cartilage extract component may be any suitable kind of cartilage or biochemical component thereof, typically proteoglycans (consisting of protein and mucopolysaccharides), whether extractable from the cartilage or preparable synthetically. The term "cartilage or cartilage extract" is intended to include components that may also be found in other tissue containing connective tissue, e.g. skin or hide, and may be extracted therefrom. The cartilage is preferably selected from bovine cartilage, porcine cartilage, shark cartilage, squid cartilage, chicken cartilage, salmon cartilage. The cartilage extract is preferably selected from extract components of such cartilages or from similar components extracted from other sources; chondroitin sulphate; keratan sulphate; hyaluronic acid; dermatan sulphate; or mixtures thereof, obtained from cartilage or from other sources. A particularly preferred cartilage component is shark cartilage and extract thereof. If cartilage is used, it may typically be used in the form of dried, e.g. lyophilised, comminuted cartilage. Useful extracts of the above mentioned types of cartilage or other tissue containing the appropriate components may typically be prepared through partial enzymatic proteolytic hydrolysis of cooked tissue followed by filtration and drying of the hydrolysate, e.g. through spray drying or lyophilisation. Such extracts have the advantage of being partially or fully soluble in aqueous media.

In the composition of the invention, the hydrophilic antioxidant is preferably one which exhibits an antioxidant activity $CI_{50}$ of at least $5 \times 10^{-7}$ for scavenging R'/ROO' radicals in lipid peroxidation of an unsaturated phospholipid in an aqueous medium, cf. the above.

The hydrophilic antioxidant component is preferably selected from the group consisting of ascorbic acid (vitamin C) or pharmaceutically acceptable salts thereof as well as plant extracts containing ascorbic acid (e.g. extracts of citrus other fruits), and polyphenols of catechic or flavonoid structure and oligomers thereof, and mixtures thereof. Useful exam-

ples of such polyphenols of catechic or flavonoid structure and oligomers thereof are flavonoids such as cathecin, epicathecin and gallic acid; oligomers (dimers, trimers, tetramers, pentamers, hexamers and heptamers) of catechin or epicatechin, also known as procyanidins; as well as gallates of such flavonoids and oligomers. The catechin and epicatechin oligomers are usually formed through bonds between the 8- and 4-positions or between the 6- and 4-positions of the benzopyran nucleus. Other useful examples of the hydrophilic antioxidant component is the silymarin group of flavolignan compounds or one of the components thereof (silybin, silydianin, silychristin and isosilybin), proanthocyanin A2, and procyanidole oligomers extracted from pine bark, seeds of *Vitis vinifera,* leaves of *Camelia sinensis,* seeds of *Aesculus hippocastanum,* seeds and/or leaves of *Gingo biloba* and fruits and/or seeds of *Cardus marianum,* fruits and/or leaves of *Vaccinium mirtillus,* seeds of *Silybum marianum*, or mixtures thereof.

A particularly preferred hydrophilic antioxidant is an extract from grape seed, i.e. seeds of *Vitis vinifera*, said extract typically being obtained by extracting grape seeds using organic solvents such as acetone and/or ethyl acetate or the like, evaporating the solvents, re-dissolving the residue in water, and filtering and drying the filtrate, e.g. by spray drying or lyophilisation. In a particularly preferred embodiment, such an extract typically contains up to 25% w/w of catechin, epicatechin and galllic acid; up to 90% w/w of epicatechin dimer, trimer and/or tetramer, and/or gallates thereof; and up to 10% w/w of epicatechin pentamer, hexamer and/or heptamer, and/or gallates thereof.

In an especially preferred embodiment, the composition according to the invention comprises

i) an extract of shark cartilage
ii) grape seed extract and
iii) lycopene.

Such a composition may typically contain 1-80% w/w of extract of shark cartilage, 0.1-75% w/w of grape seed extract, and 0.002-25% w/w of lycopene. The extract of shark cartilage is typically one which contains 5-100% w/w of chondroitin sulphate (usually consisting of a mixture of chondroitin sulphate C, chondroitin sulphate A and chondroitin sulphate E). The grape seed extract typically contains 50-100% w/w of procyanidin.

In compositions according to the invention, the weight ratio between the hydrophilic and the lipophilic antioxidant is preferably in the range from about 1:1 to about 200:1, preferably from about 2:1 to about 100:1, in particular from about 5:1 to about 50:1, especially from about 5:1 to about 20:1, such as about 10:1.

The compositions for oral use according to the invention may suitably be in the form of a solid dosage form such as tablets, lozenges, powders, granules and hard and soft gelatine capsules; or in the form of a liquid dosage form selected from solutions, suspension, tonics and syrups. Such dosage forms may be prepared in a manner well known in the art of pharmaceutical technology and may contain one or more excipients which may be any of those commonly used within the art. For solid compositions, conventional non-toxic solid excipients may be used including, but not limited to, e.g. pharmaceutical grades of mannitol, lactose, starch, soybean fibre, magnesium stearate, sodium saccahrin, talc, cellulose such as microcrystalline cellulose, glucose, saccharose, silicon dioxide, magnesium carbonate or the like. Liquid dosage forms may be obtained by dissolving, dispersing etc. the active components and an optional pharmaceutical adjuvant in an excipient such as water or water-based liquids such a juices, oil or alcohol, in order to form a solution or suspension. If desired, the oral composition according to the invention may also contain minor amounts of non-toxic additives known in the art such as wetting or emulsifying agents, buffers, or the like. Such dosage forms may be formulated in accordance with principles well known in the art, cf. also e.g. *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Easton, Pennsylvania, 15th Edition, 1975; or Martindale, *The Extra Pharmacopeia*, The Royal Pharmaceutical Society of Great Britain, 31th Edition, 1996.

The compositions typically contain about 50-3000 mg cartilage component, about 5-500 mg of the hydrophilic antioxidant, and 0,1-200 mg of the lipophilic antioxidant per dosage unit, or 55-3700 mg of the total mixture of cartilage component, hydrophilic antioxidant and lipophilic antioxidant.

The compositions may be packaged according to principles well known in the art such as in tablet containers, blister packs or bottles. In the event that one of components is sensitive to light which in particular is the case if the lipophilic antioxidant is lycopene, it is advisable to shield the composition from light. In the case of e.g. blister packs, this may suitably be attained if the blister packs are of the well-known type formed from two sheets of aluminium foil and aluminium coated plastic foil, respectively, such as a shaped (form-

ing depressions) sheet of a plastic/aluminium laminate (e.g. a laminate of PVC, aluminium and orientated polyamide foils) and a thin, optionally varnished aluminium foil, respectively.

In a second aspect, the invention concerns a composition as defined above for use as a cosmetic composition, a food supplement, a pharmaceutical or a dietetic.

In these uses, a typical daily dosage for an average adult person is from 55 to 3700 mg of the above defined mixture of cartilage component, hydrophilic antioxidant and lipophilic antioxidant, such as from 70 to 1000 mg. The administration may take place in one daily dose or in divided doses up to four times a day.

The invention is illustrated further by the following, non-limiting examples.

EXAMPLE 1

A composition according to the invention was prepared using the following ingredients mixed in the stated proportions (the amounts given are per final dosage unit):

105 mg of shark cartilage extract (prepared through enzymatic proteolytic hydrolysis of shark cartilage, filtration and spry drying of the hydrolysate);
100 mg plant extracts (containing ca. 1,5 mg lycopene extracted from tomatoes and ca. 14 mg flavonoids extracted from grape seeds, the remainder being soy fiber, tomato oil and silicon dioxide; Alextan® from Indena, Milano, Italy);
30 mg Acerola extract (containing ca. 7,5 mg ascorbic acid, the remainder being Acerola constituents and maltodextrin);
67 mg microcrystalline cellulose
4 mg silicon dioxide (particle size 2.4-3.6 μm).

The mixing was performed in a Lödige mixer for 6 minutes and subsequently in a conical mixer. The powder mixture was pressed into tablets with a weight of 305 mg.

EXAMPLE 2

The composition prepared in Example 1 can be tested on a group of healthy volunteers to determine changes in skin texture and elasticity. The test is typically carried out by re-

cording, at day 0, standard photographs of the face each individual subject at the beginning of the trial and having th subject fill out a questionnair asking the subject's opinion of various qualities of his/her skin in the face and upper chest. This procedure is repeated on days 30, 60 and 90. Selected test subjects are to continue and are subjected to the same procedure again at days 180, 270 and 360.

CLAIMS

1. A composition for oral use, said composition comprising

i) cartilage or an extract thereof,

ii) a hydrophilic antioxidant, and

iii) a lipophilic antioxidant exhibiting an antioxidant activity $CI_{50}$ of at least $1.1 \times 10^{-7}$ for scavenging $R^{\cdot}/ROO^{\cdot}$ radicals in lipid peroxidation of an unsaturated phospholipid in an aqueous medium.

2. A composition according to claim 1 wherein the lipophilic antioxidant exhibits an antioxidant activity $CI_{50}$ of at least $1.2 \times 10^{-7}$ for scavenging $R^{\cdot}/ROO^{\cdot}$ radicals in lipid peroxidation of an unsaturated phospholipid in an aqueous medium, preferably at least $1.3 \times 10^{-7}$, more preferably at least $1.4 \times 10^{-7}$, particularly at least $1.5 \times 10^{-7}$, more particularly at least $1.6 \times 10^{-7}$, especially at least $1.7 \times 10^{-7}$, such as at least $1.8 \times 10^{-7}$.

3. A composition according to claim 1 or 2 wherein the lipophilic antioxidant is lycopene, optionally in combination with another lipophilic antioxidant.

4. A composition according to any of claims 1-3, wherein the cartilage or an extract thereof is selected from bovine cartilage, porcine cartilage, shark cartilage, squid cartilage, chicken cartilage, salmon cartilage, or extracts thereof; chondroitin sulphate; keratan sulphate; hyaluronic acid; dermatan sulphate; or mixtures thereof.

5. A composition according to any of claims 1-4, wherein the hydrophilic antioxidant is selected from the group consisting of ascorbic acid (vitamin C) or pharmaceutically acceptable salts thereof, and polyphenols of catechic or flavonoid structure and oligomers thereof, and mixtures thereof.

6. A composition according to claim 5, wherein the hydrophilic antioxidant is selected from silymarin or one of the components thereof (silybin, silydianin, silychristin and isosilybin), proanthocyanin A2, procyanidole oligomers extracted from pine bark, *Vitis vinifera, Camelia sinensis, Aesculus hippocastanum, Gingo biloba* and *Cardus marianum, Vaccinium mirtillus, Silybum marianum*, or mixtures thereof.

16. A composition according to claim 13, wherein the grap seed extract contains 50-100% w/w of procyanidin.

17. A composition according to any of the preceding claims, wherein the weight ratio between the hydrophilic and the lipophilic antioxidant is in the range from about 1:1 to about 200:1, preferably from 2:1 to 100:1, in particular from 5:1 to 50:1, especially from 5:1 to 20:1, such as about 10:1.

18. A composition according to any of the preceding claims in the form of a solid dosage form selected from tablets, powders, granules and hard and soft gelatine capsules; or in the form of a liquid dosage form selected from solutions, suspension, tonics and syrups.

19. A composition according to any of the preceding claims for use as a cosmetic composition, a food or food supplement, a pharmaceutical or a dietetic.

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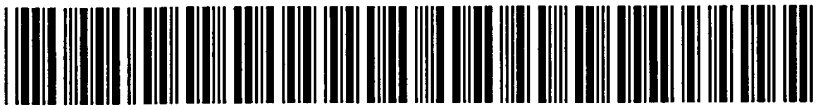

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